Conferences and Reviews

Hepatitis C Virus
A Review

EDDIE TANG, MD, *Los Angeles, California*

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LINDA HAWES CLEVER, MD
Editor

Hepatitis C virus has been shown to be responsible for most cases of posttransfusion hepatitis, as well as for sporadic non-A, non-B viral hepatitis. Hepatitis C virus has also been implicated in the development of primary hepatocellular carcinoma, autoimmune hepatitis, and fulminant viral hepatitis. Although the role of the parenteral transmission of hepatitis C virus is well established, its route of transmission in cases of sporadic infection remains unclear. Sexual transmission is suspected but not confirmed. Recent work regarding treatment has shown interferon alfa to be effective, but the discontinuation of therapy is associated with a 50% relapse rate.

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Hepatitis develops in nearly 10% of all blood transfusion recipients.[1] In the United States alone, this translates into more than 15,000 new cases each year of cirrhosis caused by transfusion.[2] In the early 1970s, posttransfusion hepatitis was thought to be due to the hepatitis B virus (HBV). With the development of sensitive assays for hepatitis B surface antigen (HBsAg), it became clear that HBV was responsible for less than 25% of such cases.[1] Serologic tests for hepatitis A virus (HAV) likewise showed HAV to be rarely responsible. The term non-A, non-B hepatitis was, therefore, adopted for these cases. With the introduction of routine screening of blood donors for serum HBsAg, 90% to 95% of cases of posttransfusion hepatitis have become characterized as non-A, non-B hepatitis.[1]

Numerous attempts have been made to find the cause of non-A, non-B hepatitis. Until recently, however, the responsible agent had remained elusive, and no screening tests were available. As a result, surrogate markers associated with an increased risk for non-A, non-B hepatitis transmission were identified and used to screen blood donations. The markers found to be useful were elevations of serum alanine aminotransferase levels and the presence of anti-hepatitis B core antibody (HBcAb).

Breakthrough

In 1988, workers at Chiron Corporation announced the discovery of viral antigens specific for posttransfusion non-A, non-B hepatitis.[3] They isolated all nucleic acid from known infectious serum and, from this pool, formed complementary DNA fragments using reverse transcriptase with random primers. This process yielded approximately 6 million sequences complementary to random segments of nucleic acid found in the infectious serum. These sequences were then individually inserted into phage vectors and expressed in *Escherichia coli*. Each resulting polypeptide was tested with serum from patients with chronic non-A, non-B hepatitis to detect reactivity with serum antibodies. Each polypeptide was also screened with control specimens of noninfected serum. After about a million such specimens were screened, one was found to react with antibodies in infected serum but not with control serum.

The complementary DNA fragment used to generate this polypeptide was then used as a hybridization probe to extract the original nucleic acid from which the fragment was generated. In this way, the entire genome of the suspected agent was identified. It was found to be composed of a single positively stranded RNA about 10,000 nucleotides long. From homologies found within the genomic structure, it appeared that this agent, now termed hepatitis C virus (HCV), was related to the family of togaviruses or flaviviruses. This identification was consistent with the 30- to 60-nm size of the virus,[4] as determined by filtration studies, and with the presence of a viral envelope, as recognized by the agent's sensitivity to chloroform.[5]

To develop a serologic test for HCV, a segment of DNA based on the one that produced the original reactive polypeptide was inserted into a plasmid. This viral segment, coding for 363 amino acids from the nonstructural portion of the viral genome, was first ligated with the gene for superoxide dismutase before insertion in order to increase polypeptide production. The resulting protein sequence was expressed in yeast culture and used as the antigen basis for radioimmunoassay and enzyme-linked immunosorbent assay (ELISA).

Epidemiology

To assess the role of the newly identified HCV as the etiologic agent for posttransfusion non-A, non-B hepatitis, Van Der Poel and colleagues in 1989 studied nine cardiac surgery patients with documented posttransfusion non-A, non-B hepatitis and nine matched controls.[5] Serial serum specimens from patients having cardiac surgery showed that four seroconverted, as compared with none of the nine con-

From the University of Southern California School of Medicine, Los Angeles.
Reprint requests to Eddie Tang, MD, 2141 Alta St, #10, Los Angeles, CA 90031.

ABBREVIATIONS USED IN TEXT

ALT = alanine aminotransferase
CID = chimp infectious dose
ELISA = enzyme-linked immunosorbent assay
HAV = hepatitis A virus
HBcAb = hepatitis B core antibody
HBsAg = hepatitis B surface antigen
HBV = hepatitis B virus
HCV = hepatitis C virus
HIV = human immunodeficiency virus

trols. Furthermore, seven of the nine surgical patients had donor serum that was HCV-positive. In a prospective study of 34 patients with posttransfusion non-A, non-B hepatitis in Spain, 4 (12%) became seropositive by 8 weeks, 15 (44%) by 28 weeks, and 27 (79%) by 52 weeks.[6] Other studies also have found the prevalence of HCV-positivity in cases of posttransfusion non-A, non-B hepatitis to be about 80%.[7,8]

Various factors appear to affect the rate of HCV seroconversion. First, a long time lag before seroconversion is observed using the currently available tests. Studies in the United States have shown conversion rates of 32%, 71%, and 81% in patients with acute disease, disease of an indeterminate duration, and chronic disease, respectively.[6] The mean seroconversion time is said to be 15 weeks after the clinical occurrence of hepatitis, with a range of 4 to 32 weeks.[4] Seroconversion occurring more than a year after exposure has been reported.[9] Such long time lags have important implications concerning follow-up in any seroprevalence study. Seroconversion rates also appear to be lower in patients with self-limiting disease, as opposed to those that progress to a chronic course. In a study in Japan, 20 of 22 patients with non-A, non-B hepatitis that progressed to chronicity were HCV-positive, while only 5 of 11 patients with self-limiting infections were positive ($P < .02$).[4] Moreover, the antibody response in those patients with self-limiting disease who do seroconvert is often more labile, declining and sometimes disappearing within a few years, whereas persistent levels are maintained in patients with chronic disease.[4] Along with the long time lag of seroconversion, this difference may indicate that there is insufficient antigen in patients with self-limiting infections to stimulate a detectable humoral response. Although it is conceivable that a different viral agent is involved, studies of animals have clearly shown that the same inoculum can lead to either chronic or self-limiting infections.[4]

Aside from its role in posttransfusion non-A, non-B hepatitis, HCV also appears to play an important role in nontransfusion-associated non-A, non-B hepatitis. This so-called sporadic form accounts for 10% to 25% of all cases of non-A, non-B hepatitis in the United Kingdom and nearly 50% in the United States.[10,11] In one US study, 34 of 59 patients (58%) with sporadic disease became HCV-positive at some stage after the occurrence of disease.[4] Similar studies in Germany and Italy have shown rates of 72% and 71%, respectively.[8,12] These last two rates compare with the 79% and 84% rate in transfusion-associated non-A, non-B hepatitis found in the same studies.

In contrast to the high seroprevalence rates found in the previous disease processes, the rate in the general population remains about 1%. Table 1 shows the prevalence of anti-HCV-positivity in blood donors in various countries. In addition to the composite rates shown for each country, there appear to be substantial regional variations within each particular country.

TABLE 1.—*Antihepatitis C Virus (HCV) Seroprevalence by Country*

Country	Population HCV-Positive, %	Source
United States	0.6	Williams and Dodd, 1990[6]
Japan	1.5	Williams and Dodd, 1990[6]
Canada	0.3	Williams and Dodd, 1990[6]
France	0.7	Janot et al, 1989[13]
Italy	0.9	Sirchia et al, 1989[14]
Germany	0.4	Kühnl et al, 1989[15]
Africa	6.0	Williams and Dodd, 1990[6]

Hepatitis C Virus and Primary Hepatocellular Carcinoma

Besides hepatitis, hepatitis C virus has been associated with other disease processes. The most important of these is primary hepatocellular carcinoma. Colombo and co-workers in 1989 reported the frequency of HCV-positivity in a group of patients with HBsAg-negative hepatocellular carcinoma to be 70%.[12] Likewise, Bruix and associates found an HCV-positivity rate of 81.4% in a group of patients with cryptogenic cirrhosis and hepatocellular carcinoma.[16] This association between HCV and hepatocellular carcinoma is usually recognized as the progression of chronic non-A, non-B hepatitis to cirrhosis and, finally, to carcinoma. Cirrhosis is not strictly necessary for the development of hepatocellular carcinoma, however. Studies have reported HCV-positivity rates as high as 40% in patients with hepatocellular carcinoma not associated with cirrhosis.[17]

There is also evidence that HCV may act synergistically with other agents already implicated in the development of hepatocellular carcinoma. In their study, Colombo and colleagues reported that 49 of 91 (54%) patients with HBsAg-negative hepatocellular carcinoma were both anti-HCV- and anti-HBcAb-positive, but only 26 of 139 (19%) patients with chronic non-A, non-B hepatitis were positive for both ($P < .001$).[12] In this study, the HCV-positivity rate in the carcinoma group was only 16% compared with the 55% rate in the non-A, non-B hepatitis group ($P < .001$), but the prevalence of anti-HBcAb was similar in both groups (9% versus 16%, not significant). These findings suggest that a previous HBV infection may enhance the potential for HCV to cause hepatocellular carcinoma. Such a previous HBV infection is not necessary for HCV-related carcinoma to develop, however. There are well-documented cases of HCV-related hepatocellular carcinoma in which sensitive polymerase chain reaction tests have shown no HBV DNA.[4] With regard to another cause, a Spanish study of patients with alcoholic cirrhosis with and without hepatocellular carcinoma showed HCV-positivity rates of 77% and 39%, respectively ($P < .02$).[16] The mechanism by which HCV causes hepatocellular carcinoma remains unclear. Incorporation into host DNA is unlikely because HCV replication does not appear to go through a DNA intermediate.

Hepatitis C Virus and Autoimmune Hepatitis

A study of 34 patients with chronic active autoimmune hepatitis showed a 44% prevalence of anti-HCV.[10] Similarly, Fusconi and co-workers found more than 80% of patients

TABLE 2.—*Antihepatitis C Virus (HCV) Seroprevalence in Various Risk Groups for Parenteral Exposure*

Risk Factor	*Patients Studied, No.*	*HCV-Positive, %*	*Source*
Hemophilia	756	70	Esteban et al, 1989[22]; Ludlam et al, 1989[23]; Noel et al, 1989[24]; Roggendorf et al, 1989[8]
Thalassemia	40	15	Williams and Dodd, 1990[6]
Dialysis	697	20	Williams and Dodd, 1990[6]
Intravenous drug use	236	70	Esteban et al, 1989[22]; Mortimer et al, 1989[25]; Roggendorf et al, 1989[8]

with type 2 autoimmune hepatitis to be seropositive.[18] The significance of this finding is unclear. It seems to suggest that HCV may act as an inciting factor in this disease process. This is particularly intriguing because the occurrence of autoimmune hepatitis is biphasic with respect to age, with many patients diagnosed later in life.[19] Such a pattern would be consistent with an environmental inciting agent. It is hypothesized that hepatocytes damaged by HCV may expose host antigens in such a way as to elicit an autoimmune response that persists even after the HCV infection itself has resolved. More study is clearly needed. McFarlane and associates have suggested that this high rate may merely represent false-positive results.[19] This thinking is based on their finding that although 20 of 31 patients (65%) with active autoimmune hepatitis were HCV-positive, only 1 of 22 patients in remission (5%) was positive ($P < .0005$). Furthermore, the optical density values observed from their assays were closely correlated with serum globulin ($r = .8846$, $P < .0005$) and immunoglobulin G ($r = .6281$, $P < .0005$) levels, and all the seropositive patients became seronegative when retested during remission. McFarlane and colleagues hypothesize that during active disease the anti-HCV assay may be detecting immunoglobulin G nonspecifically adhering to the ELISA plates.[19]

Transmission

The role of the parenteral transmission of HCV is well characterized in transfusions of blood or blood products. Cases of vertical transmission have also been reported,[10] as have cases of transmission by human bites.[20] Table 2 lists the HCV prevalence rates for various risk groups for parenteral exposure. Although transmission has been described in such cases, it remains unclear what the risk of a small parenteral exposure is. It is known that most patients with infections have low titers of 100 to 1,000 chimp infectious doses (CID) per milliliter.[4] Titers of as high as 1 million CID per ml have been obtained from acute-phase human serum specimens, however.[4] A recent report of 110 needle-stick exposures in medical personnel documented a 3.7% transmission rate.[21]

The route of transmission in sporadic cases is unclear. Epidemiologic studies have identified multiple heterosexual partners and sexual contacts as risk factors in persons with a history of hepatitis.[26] Table 3 shows the anti-HCV prevalence rates in various social risk groups. There is an increased prevalence of HCV-positivity in female contacts of intravenous drug abusers, and homosexual men who are positive for the human immunodeficiency virus (HIV) have a higher prevalence than homosexual men who are HIV-negative. Patients with sexually transmitted diseases likewise have increased rates. Hence, it is likely that HCV is transmitted through sexual contact. The risk of transmission, however, is apparently small. A nine-year cohort study of homosexual men by Melbye and co-workers found the cumulative incidence of HCV seroconversion to be 2.5% compared with 26% for HBV and 23% for HIV.[27] This suggests that HCV is about ten times less efficiently sexually transmitted than either HIV or HBV. In this study, variables in sexual life-style correlated with anti-HBcAb status but not with anti-HCV status.[27]

Casual contact transmission has also been suggested based on a report of HCV-positivity in 2 family members of 13 patients with chronic HCV disease.[4] A larger study by Everhart and associates failed to confirm this finding, however.[11] Interestingly, in their study, Hopf and colleagues found that of 62 cases of sporadic non-A, non-B hepatitis, 32 (52%) were in health care workers.[2] Finally, a role has also been suggested for arthropod vectors,[4] based on the fact that HCV is related to the family of togaviruses or flaviviruses, both of which are arboviruses. Arboviruses are a group of viruses usually transmitted by insect bites, and yellow fever virus, another arbovirus, is well recognized as a hepatotropic virus. In one case report, an insect vector was implicated in the clinical history of a patient with sporadic non-A, non-B fulminant viral hepatitis.[28]

Pathogenesis

It is currently unknown whether the hepatitis C virus is directly cytopathic to hepatocytes, as in the case of the hepatitis A virus, or causes damage by activating the immune destruction of infected cells, as in the case of the hepatitis B virus. The widespread changes often seen in infected tissue and the relative lack of lymphocytes in areas of damage suggest that HCV is directly cytopathic. On the other hand, the possible role of HCV in the pathogenesis of autoimmune hepatitis suggests that an immune mechanism may also be operative.

Several liver histologic features in patients with HCV hepatitis are not usually found in disease of other causes.[17] First, there are focally dense aggregates of lymphocytes in the portal areas. These infiltrates are also found within the sinusoidal space, in conjunction with Kupffer's cell hyperplasia. The bile duct epithelium is often abnormal and may mimic the lesions of primary biliary hepatitis. Although reported to be the least common finding, this is said to be the most important histologic marker for progression to chronicity.[17] These histologic findings are superimposed on those common to other types of viral hepatitis.

Fagan and Williams detected 60- to 70-nm viruslike particles with envelope surface proteins budding into the cell vacuoles of infected hepatocytes.[28] Also found were rod-

TABLE 3.—*Antihepatitis C Virus (HCV) Seroprevalence in Various Social Risk Groups*

Risk Group	*Patients Studied, No.*	*HCV-Positive, %*	*Source*
Women sex partners of intravenous drug users	8	12	Zuckerman, 1989[10]
Gay men	388	4.4	Williams and Dodd, 1990[6]
HIV-positive gay men	26	8	Zuckerman, 1989[10]

HIV = human immunodeficiency virus

shaped nuclear inclusions and cytoplasmic tubular structures consistent with the identification of HCV as an arbovirus.

Clinical Course

Posttransfusion non-A, non-B hepatitis has an incubation period of about 60 days and is known for its mild and often subclinical course.[29] Although cases of fulminant hepatic failure have been reported,[28] lethargy, anorexia, and occasional nausea are the usual presentations. Approximately 40% to 75% of patients remain completely asymptomatic, however, and the infection is recognized by the discovery of elevated serum aminotransferase levels. Such levels may peak just once, but most often they tend to fluctuate and may return to normal levels between exacerbations.[29] Jaundice is reported in only about 10% of patients and is usually mild.

Progression to chronic hepatitis, as defined by elevated serum aminotransferase levels for six months or more, occurs in about half of patients with acute hepatitis.[1] There is no good way of predicting when an acute case will progress to chronic disease. Of those patients in whom chronic disease develops, 20% will show morphologic features of cirrhosis within the decade after the acute illness.[1] Such changes usually evolve slowly over a period of decades, and the reported rates of cirrhosis increase with the duration of follow-up. Reinfection following the resolution of self-limiting disease has been reported.[4] Such cases have occurred after a second challenge with a large dose of the inoculum.

There is some question regarding the prognosis of sporadic, as opposed to posttransfusion, non-A, non-B hepatitis. Several studies in the United States, Italy, and Spain have shown posttransfusion non-A, non-B hepatitis to follow a more severe course than that of sporadic non-A, non-B hepatitis. Other studies in Germany, Switzerland, and Denmark have shown the opposite, however.[2] Hopf and colleagues found that whereas only 16% of patients with posttransfusion non-A, non-B hepatitis progressed to chronic active hepatitis or cirrhosis, 55% of those with sporadic non-A, non-B hepatitis did.[2] The significance of these differences remains unclear.

Treatment

Until recently, no therapy was shown to be effective against chronic non-A, non-B hepatitis. The use of corticosteroids, as well as the antiviral agent acyclovir, was shown to be ineffective.[33] Several randomized and controlled studies have now shown interferon alfa to be effective. Davis and associates, studying 166 patients with chronic posttransfusion non-A, non-B hepatitis, showed normal or near-normal serum alanine aminotransferase (ALT) levels in 46% of patients treated with interferon alfa-2B for six months compared with 8% of untreated controls ($P < .001$).[33] On biopsy, the treated patients also showed more regression of lobular and periportal inflammation ($P < .01$ and $P < .10$, respectively). Moreover, histologic improvement was not limited to those patients with a return to normal serum ALT levels, occurring as well in 13 of 25 (52%) nonresponding patients. A similar prospective study by Schvarcz and colleagues using the same treatment regimen for nine months showed normal ALT levels in 11 of 19 (58%) treated patients, compared with none of the 9 controls ($P < .001$).[34] This response to treatment does not appear to depend on a patient's anti-HCV status.[35] Furthermore, although anti-HCV antibody titers do not appear to change during therapy, there seems to be a

TABLE 4.—*Antihepatitis C Virus (HCV) Seroprevalence by Presence of Surrogate Markers for Non-A, Non-B Hepatitis**

Marker	*Patients, No.*	*HCV-Positive, %*
High ALT level	777	2.5
Positive for HBcAb	436	5.3
Both	60	37

ALT = alanine aminotransferase, HBcAb = hepatitis B core antibody

*From Williams and Dodd.6

dramatic reduction in serum HCV-RNA content after as little as four weeks of treatment.[36]

Unfortunately, the discontinuation of therapy is associated with a high rate of relapse, as shown by elevated ALT levels. In the Davis study, 51% of responding patients had relapsed by six months,[33] and in the Schvarcz study, 64% had relapsed by six months.[34] A year after discontinuation, Di Bisceglie and co-workers found that 80% of responders had relapsed.[37] In general, those patients who relapse will respond if therapy is reinstituted.[34] Previous relapse rates do not include those patients who have a transient flare in serum ALT levels following the discontinuation of therapy.[33] Such a flare is reported to occur in as many as 36% of responders, and retreatment is not indicated in such patients.[33]

Prevention

Before the current anti-HCV assay was available, two surrogate markers were found to be associated with an increased risk of posttransfusion non-A, non-B hepatitis transmission. The routine screening of donor serum for ALT and anti-HBcAb resulted in a 50% decreased incidence of posttransfusion non-A, non-B hepatitis, with only a 5% loss in the blood supply.[6] Table 4 relates the HCV seroprevalence rates associated with each marker and shows the efficacy of screening.[6] The 37% HCV-positivity rate found when both markers are present appears to be relatively low. The cause of this lies in the long time lag associated with antibody development. Although infectious, a patient's serum may not be HCV-positive for more than six months. The current anti-HCV assays will therefore not obviate the need for surrogate marker screening.

Preliminary studies in the United States have implicated donors in the transmission of HCV hepatitis who were only HCV-positive and did not have either marker present. Investigators in Japan studied such donors and reported that of 21 recipients, non-A, non-B hepatitis developed in 16 (76%), with seroconversion occurring in 15 of those patients.[4] Of the other 5 recipients, 1 had preexisting HCV positivity, another already had chronic active hepatitis with cirrhosis at the time of transfusion, and 3 had demonstrable but nondiagnostic liver damage. Alter and associates predicted that routine donor screening would detect about 85% of transfusions capable of transmitting non-A, non-B hepatitis.[9] Those cases that are not identified would consist of recently infected donors who have yet to seroconvert and previously infected donors whose antibody responses have disappeared. We do not know whether seronegative donations from patients whose previous antibody response has disappeared remain infectious.[38]

There is currently some controversy regarding whether HCV-positive donated blood should be removed from the plasma pools used for the preparation of albumin, coagulation factors, and immunoglobulins. Workers in France have advocated exclusion,[4] but the US Food and Drug Administration has decided not to screen until more is known about the effects of screening on the safety of the derived products.[39] Screening may not decrease the amount of virus in the pool but instead merely decrease the amount of antibodies against it.[39] Heating clotting factor concentrate or treating it with solvents or detergents results in the complete absence of seroconversion.[4]

Future Developments

Intense investigation is now under way regarding the development of better tests for HCV. Newer assays still based on the currently produced antigen, such as the recombinant immunoblot assay and modifications of the existing ELISA test, promise to offer better specificity. The current antigen, representing approximately 12% of the viral genome, does not bind a neutralizing antibody, and the current anti-HCV assays cannot distinguish between infectious and noninfectious HCV-positive serum specimens. Moreover, there are questions of poor specificity of the current assays in patients with other forms of chronic liver disease and in those with monoclonal gammopathies.[18,40]

Garson and co-workers recently developed a sensitive polymerase chain reaction method for the detection of HCV RNA that appears to provide good correlation with proved infectivity.[41] Further study of the now-characterized HCV genome promises the discovery of newer tests with better sensitivities and specificities, a better response in the acute phase of disease, and a better predictive value concerning which patients will have progression to chronic disease. Such study may also provide the basis for an as-yet-unavailable confirmatory test, as well as allow the discovery of a neutralizing antibody. This in turn may lead to a vaccine for HCV disease.

Although the ability to test for the hepatitis C virus has shown it to be the dominant cause of non-A, non-B hepatitis, there are instances where HCV cannot be identified. There is good evidence for at least one other agent being responsible for this form of hepatitis.[4] This agent is distinguished from HCV by its chloroform insensitivity. There may be others as well. It is interesting to note that Arima and associates in Okayama, working independently from researchers at Chiron Corporation but using similar techniques, isolated several clones that expressed polypeptides shown in an independent laboratory to react specifically with well-documented panels of non-A, non-B hepatitis serum.[42] These clones, however, do not appear to bear a relationship to any part of the HCV genome characterized at Chiron.[4] The importance of this finding is unclear at present.

REFERENCES

1. Seeff LB, Dienstag JL: Transfusion-associated non-A, non-B hepatitis—Where do we go from here? Gastroenterology 1988; 95:530-533

2. Hopf U, Möller B, Küther D, et al: Long-term follow-up of posttransfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus (HCV). J Hepatol 1990; 10:69-76

3. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M: Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244:359-362

4. Choo QL, Weiner AJ, Overby LR, Kuo G, Houghton M, Bradley DW: Hepatitis C virus: The major causative agent of viral non-A, non-B hepatitis. Br Med Bull 1990; 46:423-441

5. Will the real hepatitis C stand up? (Editorial). Lancet 1989; 2:307-308

6. Williams AE, Dodd RY: The serology of hepatitis C virus in relation to post-transfusion hepatitis. Ann Clin Lab Sci 1990; 20:192-199

7. Kuo G, Choo QL, Alter HJ, et al: An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989; 244:362-364

8. Roggendorf M, Deinhardt F, Rasshofer R, et al: Antibodies to hepatitis C virus (Letter). Lancet 1989; 2:324-325

9. Alter HJ, Purcell RH, Shih JW, et al: Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989; 321:1494-1500

10. Zuckerman AJ: The elusive hepatitis C virus. BMJ 1989; 299:871-873

11. Everhart JE, Di Bisceglie AM, Murray LM, et al: Risk for non-A, non-B (type C) hepatitis through sexual or household contact with chronic carriers. Ann Intern Med 1990; 112:544-545

12. Colombo M, Kuo G, Choo QL, et al: Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989; 2:1006-1008

13. Janot C, Courouce AM, Mainez M: Antibodies to hepatitis C virus in French blood donors. Lancet 1989; 2:796-797

14. Sirchia G, Bellobuono A, Giovanetti A, Marconi M: Antibodies to hepatitis C virus in Italian blood donors. Lancet 1989; 2:797

15. Kühnl P, Seidl S, Stangel W, Beyer J, Sibrowski W, Flik J: Antibody to hepatitis C virus in German blood donors (Letter). Lancet 1989; 2:324

16. Bruix J, Barrera JM, Calvet X, et al: Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989; 2:1004-1006

17. Underwood JC: Hepatitis C virus and transfusion transmitted liver disease: Review. J Clin Pathol 1990; 43:445-447

18. Fusconi M, Lenzi M, Ballardini G, et al: Anti-HCV testing in autoimmune hepatitis and primary biliary cirrhosis (Letter). Lancet 1990; 336:823-824

19. McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vergani D, Williams R: Hepatitis C virus antibodies in chronic active hepatitis: Pathogenetic factor or false-positive results? Lancet 1990; 335:754-757

20. Dusheiko GM, Smith M, Scheuer PJ: Hepatitis C virus transmitted by human bite (Letter). Lancet 1990; 336:503-504

21. Cotton P: Tests help nail down HCV—But not entirely. JAMA 1991; 3:312

22. Esteban JI, Esteban R, Viladomiu L, et al: Hepatitus C virus antibodies among risk groups in Spain. Lancet 1989; 2:294-297

23. Ludlam CA, Chapman D, Cohen B, Litton PA: Antibodies to hepatitis C virus in haemophilia (Letter). Lancet 1989; 2:560-561

24. Noel L, Guerois C, Maisonneuve P, Verroust F, Laurian Y: Antibodies to hepatitis C virus in haemophilia (Letter). Lancet 1989; 2:560

25. Mortimer PP, Cohen BJ, Litton PA, et al: Hepatitis C virus antibody (Letter). Lancet 1989; 2:798

26. Benamouzig R, Ezratty V, Chaussade S: Risk for type C hepatitis through sexual contact. Ann Intern Med 1990; 113:638

27. Melbye M, Biggar RJ, Wantzin P, Krogsgaard K, Ebbesen P, Becker NG: Sexual transmission of hepatitis C virus: Cohort study (1981-1989) among European homosexual men. BMJ 1990; 301:210-212

28. Fagan EA, Williams R: Fulminant viral hepatitis. Br Med Bull 1990; 46:462-480

29. McIntyre N: Clinical presentation of acute viral hepatitis. Br Med Bull 1990; 46:533-547

30. Dienstag JL: Non-A, non-B hepatitis: I. Recognition, epidemiology, and clinical features. Gastroenterology 1983; 85:439-462

31. Koretz RL, Stone O, Gitnick GL: The long-term course of non-A, non-B post-transfusion hepatitis. Gastroenterology 1980; 79:893-898

32. Jové J, Sánchez-Tapias JM, Bruguera M, et al: Post-transfusion vs. sporadic non-A, non-B chronic hepatitis—A clinico-pathological and evolutive study. Liver 1988; 8:42-47

33. Davis GL, Balart LA, Schiff ER, et al: Treatment of chronic hepatitis with recombinant interferon alfa—A multicenter randomized, controlled trial. N Engl J Med 1989; 321:1501-1506

34. Schvarcz R, Weiland O, Wejstål R, Norkrans G, Frydén A, Foberg U: A randomized controlled open study of interferon alpha-2b treatment of chronic non-A, non-B posttransfusion hepatitis: No correlation of outcome to presence of hepatitis C virus antibodies. Scand J Infect Dis 1989; 21:617-625

35. Marcellin P, Giostra E, Boyer N, Loriot MA, Martinot-Peignoux M, Benhamou JP: Is the response to recombinant alpha interferon related to the presence of antibodies to hepatitis C virus in patients with chronic non-A, non-B hepatitis? J Hepatol 1990; 11:77-79

36. Kanai K, Iwata K, Nakao K, Kako M, Okamoto H: Suppression of hepatitis C virus RNA by interferon-alpha (Letter). Lancet 1990; 336:245

37. Di Bisceglie AM, Martin P, Kassianides C, et al: Recombinant interferon alpha therapy for chronic hepatitis C—A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989; 321:1506-1510

38. Stevens CE, Taylor PE, Pindyck J, et al: Epidemiology of hepatitis C virus—A preliminary study in volunteer blood donors. JAMA 1990; 263:49-53

39. Finlayson JS, Tankersley DL: Anti-HCV screening and plasma fractionation: The case against (Letter; Comment). Lancet 1990; 335:1274-1275

40. Gray JJ, Wreghitt TG, Friend PJ, Wight DG, Sundaresan V, Calne RY: Differentiation between specific and non-specific hepatitis C antibodies in chronic liver disease. Lancet 1990; 335:609-610

41. Hepatitis C virus upstanding (Editorial). Lancet 1990; 335:1431-1432

42. Arima T, Shimomura H, Tsuji T, et al: Serum RNA associated with blood-transmitted non-A, non-B hepatitis. Hepatology 1988; 8:1275